Captan

• Intended Use

For detection of captan in water samples which require no sample preparation. Application bulletins for the detection of captan in specific foods and crops are available. To develop additional methods refer to the Sample Information section, below.

• Principle

The Captan RaPID Assay® applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of captan. The sample to be tested is added, along with an enzyme conjugate, to a disposable test tube, followed by paramagnetic particles with antibodies specific to captan attached. Both the captan (which may be in the sample) and the enzyme labeled captan (the enzyme conjugate) compete for antibody binding sites on the magnetic particles. At the end of an incubation period, a magnetic field is applied to hold the paramagnetic particles (with captan and labeled captan analog bound to the antibodies on the particles, in proportion to their original concentration) in the tube and allow the unbound reagents to be decanted. After decanting, the particles are washed with Washing Solution.

The presence of captan is detected by adding the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme-labeled captan analog bound to the captan antibody catalyzes the conversion of the substrate/ chromogen mixture to a colored product. After an incubation period, the reaction is stopped and stabilized by the addition of acid. Since the labeled captan (conjugate) was in competition with the unlabeled captan (sample) for the antibody sites, **the color developed is inversely proportional to the concentration of captan in the sample** .

• Reagents

1. Captan Antibody Coupled Paramagnetic Particles
The captan antibody (rabbit anti-captan) is covalently bound to paramagnetic particles, which are suspended in buffered saline containing preservative and stabilizers.
30 test kit: one 20 mL vial
100 test kit: one 65 mL vial

2. Captan Enzyme Conjugate
The horseradish peroxidase (HRP) labeled captan analog is diluted in buffered saline containing preservative and stabilizers.
30 test kit: one 10 mL vial
100 test kit: one 35 mL vial

3. Captan Standards
Three levels of calibrators equivalent to 0.08, 0.50 and 3.00 ppm of captan in buffered saline containing preservative and stabilizers are supplied. Each vial contains 2.0 mL.

4. Control
A level approximately equivalent to 0.20 ppm of captan in buffered saline with preservative and stabilizers. A 2.0 mL volume is supplied in one vial.

5. Diluent/Zero Standard
Buffered saline containing preservative and stabilizers without any detectable captan.
30 test kit: one 10 mL vial
100 test kit: one 35 mL vial

6. Color Solution
A solution of hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine in an organic base.
30 test kit: one 20 mL vial
100 test kit: one 65 mL vial

7. Stopping Solution
A solution of sulfuric acid (0.5%).
30 test kit: one 20 mL vial
100 test kit: one 60 mL vial

8. Washing Solution
Preserved deionized water.
30 test kit: one 70 mL vial
100 test kit: one 250 mL vial

9. Test Tubes
Polystyrene tubes (36) are packaged in a box.
30 test kit: one 36 tube box
100 test kit: three 36 tube boxes

• Reagent Storage and Stability

Store all reagents at 2-8°C. Do not freeze. Reagents may be used until the expiration date on the box. *The test tubes require no special storage condition and may be stored separately from the reagents to conserve refrigerator space.*

Consult state, local and federal regulations for proper disposal of all reagents.

• Materials Required but Not Provided

In addition to the reagents provided, the following items are essential for the performance of the test:

Pipets* Precision pipets capable of delivering 200, 250 and 500 uL and a 1.0 mL repeating pipet.

Vortex Mixer* Thermolyne Maxi Mix, Scientific Industries Vortex Genie, or equivalent

Magnetic Separation Rack*

RPA-I™ RaPID Analyzer* or equivalent photometer capable of readings at 450 nm

* These items are available from Strategic Diagnostics Inc.

• Sample Information

To reduce the rate of captan hydrolysis, it is recommended that once samples are prepared in diluent, they be kept on ice and assayed as soon as possible. All other kit reagents, including the standards and control, must be at room temperature prior to use.

To develop additional sample preparation methods several factors should be considered including: 1) Particulates - Samples containing gross particulates should be filtered. Often coarse filter paper is sufficient. 2) Solvents - Immounoassays are biological assays and can be adversely affected by solvents. Information on tolerated concentrations of solvents is available from SDI. 3) Extraction procedures - A good extraction procedure is essential for good recovery results.

Proper dilution of samples and/or other modifications to the samples may be required. Dilution of samples should be made using an appropriate amount of Diluent/Zero Standard or SampleDiluent. All diluted samples must be mixed thoroughly before assaying. **Technical assistance on developing and validating application methods is available by contacting Strategic Diagnostics Inc.**

• Reagent Preparation

All reagents must be allowed to come to room temperature and the antibody coupled paramagnetic particles should be mixed thoroughly before use.

• Procedural Notes and Precautions

As with all immunoassays, a consistent technique is the key to optimal performance. To obtain the greatest precision, be sure to treat each tube in an identical manner.

Add reagents directly to the bottom of the tube while **avoiding contact between the reagents and the pipet tip**. This will help assure consistent quantities of reagent in the test mixture.

Avoid cross-contaminations and carryover of reagents by using clean pipets for each sample addition and by avoiding contact between reagent droplets on the tubes and pipet tips.

Avoid foam formation during vortexing.

The magnetic separation rack consists of two parts: an upper rack which will securely hold the test tubes and a lower separator which contains the magnets used to attract the antibody coupled paramagnetic particles. During incubations the upper rack is removed from the lower separator so that the paramagnetic particles remain suspended during the incubation. **For separation steps, the rack and the separator are combined to pull the paramagnetic particles to the sides of the tubes**.

To obtain optimum assay precision, it is important to perform the separation steps carefully and consistently. Decant the rack by slowly inverting away from the operator using a smooth turning action so the liquid flows consistently along only one side of the test tube. While still inverted, place the rack on an absorbent pad and allow to drain. Lifting the rack and replacing gently onto the pad several times will ensure complete removal of the liquid from the rim of the tube (technique is demonstrated on training video, available from Strategic Diagnostics Inc.).

Mix the antibody coupled paramagnetic particles just prior to pipetting.

Do not use any reagents beyond their stated shelf life.

Avoid contact of Stopping Solution (sulfuric acid) with skin and mucous membranes. If this reagent comes in contact with skin, wash with water.

• Limitations

The Captan RaPID Assay will detect captan and related compounds to different degrees. Refer to specificity table for data on several of the phthalimide fungicides. The Captan RaPID Assay kit provides screening results. As with any analytical technique (GC, HPLC, etc...) positive results requiring some action should be confirmed by an alternative method.

The total time required for pipetting the magnetic particles should be kept to two (2) minutes or less, therefore the total number of tubes that can be assayed in a run should be adjusted accordingly.

• Quality Control

A control solution equivalent to approximately 0.20 ppm of captan is provided with the Captan RaPID Assay kit. It is recommended that it be included in every run and treated in the same manner as unknown samples. Acceptable limits should be established by each laboratory.

• Assay Procedure

Read Reagent Preparation, Procedural Notes and Precautions before proceeding.

1. Label test tubes for standards, control, and samples.

Tube

Number	Contents of Tube
1,2	Diluent/Zero Standard, 0 ppm
3,4	Standard 1, 0.08 ppm
5,6	Standard 2, 0.50 ppm
7,8	Standard 3, 3.00 ppm
9	Control
10	Sample 1
11	Sample 2
12	Sample 3

2. Add 200 uL of the appropriate standard, control, or sample.
3. Add 250 uL of Captan Enzyme Conjugate to each tube.
4. Mix the Captan Antibody Coupled Paramagnetic Particles thoroughly and add 500 uL to each tube.
5. Vortex for 1 to 2 seconds minimizing foaming.
6. Incubate for 30 minutes at room temperature.
7. Separate in the Magnetic Separation Rack for **two (2) minutes**.
8. Decant and **gently** blot all tubes briefly in a consistent manner.
9. Add 1 mL of Washing Solution to each tube and allow them to remain in the magnetic separation unit for **two (2) minutes**.
10. Decant and **gently** blot all tubes briefly in a consistent manner.
11. Repeat Steps 9 and 10 an additional time.
12. Remove the rack from the separator and add 500 uL of Color Solution to each tube.
13. Vortex for 1 to 2 seconds minimizing foaming.
14. Incubate for 20 minutes at room temperature.
15. Add 500 uL of Stopping Solution to each tube.
16. Add 1 mL Washing Solution to a clean test tube. Use as blank in Step 17.
17. Read results at 450 nm within 15 minutes after adding the Stopping Solution.

• Results

Manual Calculations

1. Calculate the mean absorbance value for each of the standards.
2. Calculate the %B/Bo for each standard by dividing the mean absorbance value for the standard by the mean absorbance value for the Diluent/Zero Standard.
3. Construct a standard curve by plotting the %B/Bo for each standard on vertical logit (Y) axis versus the corresponding Captan concentration on horizontal logarithmic (X) axis on the graph paper provided.
4. %B/Bo for controls and samples will then yield levels in ppm of Captan by interpolation using the standard curve.

(Contact SDI for detailed application information on specific photometers.)

RPA-I RaPID Analyzer

Using the RPA-I RaPID Analyzer, calibration curves can be automatically calculated and stored. Refer to the RPA-I operating manual for detailed instructions. To obtain results from the Captan RaPID Assay on the RPA-I the following parameter settings are recommended:

Data Reduct	:	Lin. Regression
Xformation	:	Ln/LogitB
Read Mode	:	Absorbance
Wavelength	:	450 nm
Units	:	PPM
# Rgt Blk	:	0

Calibrators:

# of Cals	:	4
# of Reps	:	2

Concentrations:

#1:	0.00	PPM
#2:	0.08	PPM
#3:	0.50	PPM
#4:	3.00	PPM

Range	:	0.01 - 3.00
Correlation	:	0.990
Rep. %CV	:	10%

• Expected Results

Comparison of the Captan RaPID Assay to gas chromatograpy (GC) and other traditional methods is available for some applications. See individual application notes.

• Performance Data

Precision

The following results were obtained:

Control	1	2	3
Replicates	10	10	10
Days	5	5	5
n	50	50	50
Mean (ppm)	0.10	0.59	2.31
% CV (within assay)	19.7	9.5	11.3
% CV (between assay)	16.0	8.8	10.7

Sensitivity

The Captan RaPID Assay has an estimated minimum detectable concentration, based on a 90% B/Bo of 0.01 ppm.

Recovery

Water was spiked with captan, frozen over dry ice/methanol and assayed, four times in duplicate immediately after thawing using the Captan RaPID Assay. The following results were obtained:

Amount of Captan Added (ppm)	Recovery Mean (ppm)	Recovery S.D. (ppm)	Recovery %
0.25	0.27	0.05	108
0.50	0.54	0.08	108
1.00	1.16	0.20	116
Average			111

Specificity

The cross-reactivity of the Captan RaPID Assay for various phthalimide fungicides and metabolites can be expressed as the least detectable dose (LDD) which is estimated at 90% B/Bo, or as the dose required to displace 50% (50% B/Bo).

Compound	LDD (ppm)	50% B/Bo (ppm)
Captan	0.01	0.42
Captafol	1.00	8.4
Folpet	8.60	>10
Tetrahydrophthalimide	10.00	>10
Phthalimide	>10.00	>10
Metolachlor	2.20	>10
Propachlor	5.00	>10
Carbaryl	5.00	>10
Alachlor	10.00	>10

The following compounds demonstrated no reactivity in the Captan RaPID Assay at concentrations up to 10 ppm: aldicarb, aldicarb sulfoxide, aldicarb sulfone, ametryn, atrazine, benomyl, butylate, carbofuran, cyanazine, 2,4-D, desethylatrazine, 1,3-dichloropropene, dinoseb, MCPA, methomyl, metribuzin, pentachlorophenol, phosphamidon, picloram, procymidone, prometron, prometryn, propazine, terbufos, terbutryn, terbutylazine, thiabendazole, and thiophanate-methyl.

• Assistance

For ordering or technical assistance contact:
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• Availability

Strategic Diagnostics Inc.
Captan RaPID Assay
100 Test Kit
Captan Sample Diluent

U.S. Patent No. 5,411,869